Small Animal Models of Plague

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Early Animal Modeling: N.B. Yersin (1898)

- Isolated a live attenuated vaccine strain virulent in rats but not in five *Macaca* species
- Human volunteer (Dr. Yersin) had only transient fever in response to the vaccine

Conclusion: Susceptibility of *Macaca* species more closely resembles that of man than the rat

Small Animal Models of Plague

- COMMON MODELS
  - Mouse
  - Guinea pig
  - Rat
- OTHER MODELS
  - Ground squirrels, rock squirrels
  - Multimammate mouse
  - Suslick
  - Voles
  - Marmots
  - Lagomorphs
  - Domestic cats

Mouse Model of Plague

- Accepted model of bubonic and pneumonic plague
- Pathology resembles that of human disease
- Desirable in terms of handling, space, and expense
- Well-established active and passive immunization models
- Useful for non-traditional vaccine strategies
- LD50 s.c. ~1-10 CFU, by aerosol 10^4-10^5 CFU

Parenteral Challenge of Mice

- Within a few hours, organisms are carried to the regional lymph nodes and transferred to the thoracic duct and the bloodstream
- Bacteremia may ensue within 6-12 hours, seeding the liver, spleen and bone marrow
- After replication in these organs, a terminal bacteremia occurs

Meyer (1949)

Pneumonic Plague in Mice

- Aerosol with small particles induces primary pneumonic plague
- Aerosol with variable particle sizes may yield disease characterized by cervical buboes and septicemia rather than pneumonic disease
- Pneumonic disease has also been observed in animals infected by intranasal installation

Meyer and Larson (1960)

Pneumonic Plague in Mice

- 12-24 h: Focal cellular infiltration of the peribronchial lymphatics
- 24-36 hours: exudate in alveoli containing numerous bacteria/ lobular bronchial pneumonia
- Within 36 h: septicemia, organisms readily cultured from blood, spleen, and bone marrow.
- Little evidence of cross infection between cagemates

Meyer, Quan, and Larson 1947

Vaccines Developed in the Mouse Model

Traditional Approaches

- Whole-cell (killed) or cell fractions
- Live attenuated
- Purified protein

Modern Approaches

- DNA-based
- Oral Salmonella-based
- Mucosal delivery

Live Attenuated Vaccines in the Mouse Model

- Results are dependent on nature of attenuation, but good protection has been demonstrated
- Residual virulence in some vaccine strains
- Inbred mice exhibit varying levels of sensitivity to attenuated strains such as EV76

Meyer (1974), Nazarova et al. (1988)

The Mouse as a Passive Protection Model: the Mouse Protection Index (MPI)

- Passive protection of mice by human test serum
- In less than 30 minutes, 1500 CFU $^{+/-}$ 500 CFU injected s.c. over right inguinal nodes
- MPI is % of mice dead/mean TTD
- MPI <10 indicates excellent protection

Efficacy of Next-generation Vaccine Candidates in Mice

- Fusion protein F1-V w/ alhydrogel gives excellent protection against parenteral (~10^7 LD_{50}s) or aerosol challenge (~800 $LD_{50)}$ in Swiss-Webster mice
- Protects against $F1^+$ and $F1^-$ strains

(Heath et al. 1998)

Vaccine Efficacy (F1 + V) in Inbred Mice

- 4 inbred mouse strains (BALB/c, CBA, C57BL6, CB6/F1) with different MHC types
- All responded with high titers (IgG1)
- Some breakthrough with CBA males
- Difficulty with male mice due to aggression
- Titers maintained in female mice for >1 year

Jones et al. (2001)

DNA Vaccines in the Mouse Model

- Early reports suggested that a prime-boost approach with DNA/ F1 protein produced good titers in inbred but not outbred mice

  Brandler et al. (1998)

- More recent studies indicate that the gene gun approach, combined with a vector that targets expression to the cytosol, may be more successful

  (Grosfield et al. 2002, Garmory et al. 2004)

Guinea Pig Model of Plague

- Sensitive to *Y. pestis* (varying LD_{50}s reported)
- Reported to have “seasonal resistance” to infection
- Variability in individual guinea pig responses
- F1 capsule an important virulence factor
- Disease resulting from s.c. infection similar to that of mice

Early Aerosol Models in the Guinea pig

- Culture suspensions “sprayed into the air”
- Necropsy showed cervical and laryngeal edema and infection (buboes), septicemia, and hemorrhage of the intestinal wall.
- Evidence of secondary lung infection rather than primary

Martini (1901, 1902), Strong and Teague (1912)

Guinea Pig Models of Pneumonic Disease

- Intratracheal installation of 5 X 10^7 organisms can produce pneumonic plague
- Intranasal installation into animals anesthetized with barbiturates flushes a small percentage of the organisms into the deeper respiratory passages
- Some guinea pigs infected by these methods have transmitted the infection to control cagemates

(Bablet and Girard 1934, Meyer et al. 1948)

Influence of Particle Size in Guinea Pig Aerosols

- Guinea pig respiratory tract does not allow particles > 4 u to reach the lungs
- Particles <1 u initiate bronchopneumonia
- Particles 10-12 u deposit in the upper airways, leading to foci in the cervical nodes followed by septicemia, but not primary pneumonia

(Druett 1956)

Guinea Pig Model of Plague

- Aerosol LD_{50} of strain CO92 similar to that of mice ($4 X10^4$)
- F1-negative strain ($pFra^-$) was attenuated
- Parenteral infection was protracted and often not dose-related

Welkos et al. (1995)

Guinea Pigs as Vaccine Models

- Respond better to live attenuated vaccines than to subunit vaccines
- Response to subunit vaccines enhanced by large antigenic mass or addition of oil-based adjuvants
- Passive protection of guinea pigs unsuccessful

Recent Guinea Pig Vaccine Studies (F1 + V in Alhydrogel)

- Anti-F1 response more variable than the mouse
- Response to F1 slower than that to V antigen
- Sera can passively protect mice
- Protective for guinea pigs at 10 LD_{50} s.c. challenge dose
- Evidence of buboe development in immunized animals, despite protection
- Failure of immunized animals to clear infection even after 26 days

Jones et al. (2003)

Live Attenuated Vaccines in Guinea Pigs

- Results are dependent on nature of attenuation, but excellent protection has been demonstrated
- Some vaccine strains demonstrated to be essentially avirulent in guinea pigs killed non-human primates

(Meyer et al. 1974)

Guinea pig model

"The guinea pig is not a suitable animal for testing plague antiserum"

"In experimental plague immunization, the reaction of the guinea pig has been unique." Spivak et al. (1957)

"The response of guinea pigs did not offer any improvement over mice in evaluating the efficacy of plague vaccines" von Metz (1971)

Rat Models of Plague

- *R. norvegicus*, *R. rattus*, *R. alexandrinas*, "Sprague-Dawley laboratory rats"
- Subcutaneous lethal dose higher than that of mice or guinea pigs (~1000-fold).
- Resistance to infection seen in laboratory rats and in captured rats from endemic and non-endemic areas
- Antibody and resistance to infection demonstrated in progeny of immunized animals

Chen and Meyer (1974), Williams et al. (1974)

Rat Model of Plague

Susceptible	Die from a relatively small dose
Partially resistant	Survive Some seroconvert and acquire immunity Nonseroconverters remain susceptible to higher dose
Resistant	Survive Do not seroconvert Remain resistant to higher doses

Rat Model of Plague

- *Rattus norvegicus* WR strain (derived from Wistar) is highly susceptible to plague regardless of age, sex, or "season of the year"
- Challenge s.c. or intranasal
- Intranasal "not as reliable" as aerosol, leads to involvement of larynx and tonsils, but "a more stringent test of vaccines than s.c. challenge"

Williams and Cavanaugh 1979

Pneumonic Plague in Mice, Guinea Pigs, and Cotton Rats

- Intranasal installation of *Y. pestis* (~10% of inoculum reaches the deeper respiratory passages)
- Primary pneumonia similar to that of man
- Mice and rats die 72-96 h
- Guinea pigs not visibly ill until “sudden death” 72-96 h
- Infection in guinea pigs confined to respiratory tract

Meyer, Quan, and Larson (1947)

Multimammate mouse model

- *Mastomys natalensis* and *M. coucha*
- Laboratory colonies established
- *M. coucha* more sensitive to *Y. pestis*
- May more closely mimic susceptibility of *Cercopithicus aethiops* to live attenuated vaccines than mice or guinea pigs
- *Naïve M. natalensis, but not M. coucha,* react to *Y. pestis* mitogens nonspecifically

Hallett (1977) Arntzen et al. (1991)

Vole model

- Resistant and sensitive groups of *Microtus californicus* bred in lab
- Nature of resistance is multigenic and may be associated with changes in phagocytic activity

Hubbert and Goldenberg (1970)

Host specificity of *Y. pestis*

- F1-negative strains are virulent in mice and NHP but significantly attenuated in guinea pigs
- Certain auxotrophs are more attenuated in guinea pigs than in mice (*aroA*, purine, asparagine)
- Some isolates from the Caucus region are virulent for mice, voles, susliks, and gerbils, but not guinea pigs

Small Animal Models of Plague

- Mouse is the best-established and accepted model
- Guinea pig has numerous drawbacks
- Other models not well-developed

"It appeared that the nature of the experimental animal was by far more essential to the results than the nature of the vaccine used" Otten 1936